5' TAGGACATTGCACCTAGGGTTTGT 3' (SEQ ID No. 2)

Cycling is performed at [96°C, 1 min]; [94°C, 1 min; 57°C, 1 min; 70°C, 2 min;] x 35; [70°C, 5 min] x 1; 4°C. Each PCR reaction is divided in two 25 ul aliquots: to one is added 5 Units of Alu 1, to the other 5 Units of Msp 1, in addition to 3 ul of the specific 10X restriction buffer. Incubation is at 37°C overnight. Electrophoresis is by PAGE 9%.

Please replace the paragraph running from page 34 to page 35 and beginning with "IL-1RN (VNTR)" with the following substitute paragraph:

IL-1RN (VNTR). The IL1-RN (VNTR) marker may be genotyped in accordance with the following procedure. As indicated above, the two alleles of the IL1-RN (+2018) marker are >97% in linkage disequilibrium with the two most frequent alleles of IL-1RN (VNTR), which are allele 1 and allele 2. The gene accession number is X64532. The oligonucleotide primers used for PCR amplification are:

5' CTCAGCAACACTCCTAT 3' (SEQ ID No. 3)

5' TCCTGGTCTGCAGGTAA 3' (SEQ ID No. 4)

Cycling is performed at [96°C, 1 min] x 1; [94°C, 1 min; 60°C, 1 min; 70°C, 2 min] x 35; [70°C, 5 min] x 1; 4°C. Electrophoresis is conducted in 2% agarose at 90V for 30 min.

Please replace the paragraph on page 35 and beginning with "IL-1A (-889)" with the following substitute paragraph:

IL-1A (-889). The IL-1A (-889) marker may be genotyped in accordance with the following procedure. McDowell et al., Arthritis Rheum. 38:221-28, 1995. One of the PCR primers has a base change to create an Nco I site when amplifying allele 1 (C at -889) to allow for RFLP

analysis. The gene accession number is X03833. The oligonucleotide primers used for PCR amplification are:

5' AAG CTT GTT CTA CCA CCT GAA CTA GGC 3' (SEQ ID No. 5)

5' TTA CAT ATG AGC CTT CCA TG 3' (SEQ ID No. 6)

Please replace the paragraph running from page 35 to page 36 and beginning with "IL-1A (+4845)" with the following substitute paragraph:

IL-1A (+4845). The IL-1A (+4845) marker may be genotyped in accordance with the following procedure. The PCR primers create an Fnu 4H1 restriction site in allele 1 to allow for RFLP analysis. The gene accession number is X03833. The oligonucleotide primers used for PCR amplification are:

5' ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA 3' (SEQ ID No. 7)

5' AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT 3' (SEQ ID No. 8)

Please replace the paragraph on page 36 and beginning with "IL-1B (-511)" with the following substitute paragraph:

IL-1B (-511). The IL-1B (-511) marker may be genotyped in accordance with the following procedure. The gene accession number is X04500. The oligonucleotide primers used for PCR amplification are:

5' TGG CAT TGA TCT GGT TCA TC 3' (SEQ ID No. 9)

5' GTT TAG GAA TCT TCC CAC TT 3' (SEQ ID No. 10)

Please replace the paragraph running from page 36 to 37 and beginning with "IL-1B (+3954)" with the following substitute paragraph:

IL-1B (+3954). The IL-1B (+3954) marker may be genotyped in accordance with the following procedure. The gene accession number is X04500. The oligonucleotide primers used for PCR amplification are:

5' CTC AGG TGT CCT CGA AGA AAT CAA A 3' (SEQ ID No. 11)

5' GCT TTT TTG CTG TGA GTC CCG 3' (SEQ ID No. 12)

Please replace the paragraph running from page 37 to 38 and beginning with "IL-1A (222/223)"; IL-1A(gz5/gz6); gaat.p33330; and Y31" with the following substitute paragraph:

IL-1A (222/223); IL-1A (gz5/gz6); gaat.p33330; and Y31. Genotyping of these markers could proceed as described in Cox et al., Am. J. Human Genet. 62:1180-88, 1998. PCRs for these markers may be carried out by using fluorescently labeled forward primers (Cruachem) in a 10 µl reaction volume containing 50 mM KCL, 10 mM Tris-HCl, pH 9.0, 1.5 mM MgCl2, 200 µM dNTPs, 25 ng of each primer, 50 ng DNA, 0.004% W-1 (Gibco-BRI), and 0.2 units Taq polymerase. The PCR conditions could be 94/ for 1 min., 55/ for 1 min., and 72/ for 1 min. for 30 cycles. One unit PERFECT MATCH (Stratagene) would be added to gz5/gz6 PCRs. The primer sequences could be as follows: for IL-1A (222/223):

5' ATGTATAGAATTCCATTCCTG 3' (SEQ ID No. 13)

5' TAAAATCAAGTGTTGATGTAG 3' (SEQ ID No. 14)

For IL-1A (gz5/gz6):

5' GGGATTACAGGCGTGAGCCACCGCG 3' (SEQ ID No. 15)

5' TTAGTATTGCTGGTAGTATTCATAT 3' (SEQ ID No. 16)

For gaat.p33330:

5' GAGGCGTGAGAATCTCAAGA 3' (SEQ ID No. 17)

5' GTGTCCTCAAGTGGATCTGG 3' (SEQ ID No. 18)

For Y31:

5' GGGCAACAGAGCAATGTTTCT 3' (SEQ ID No. 19)

5' CAGTGTGTCAGTGTACTGTT 3' (SEQ ID No. 20)

Please replace the paragraph on page 38 and beginning with "IL-1RN exon 1ic (1812); IL-1RN exon 1ic (1868); IL-1RN exon 1ic (1887); Pic (1731)" with the following substitute paragraph:

IL-1RN exon 1ic (1812); IL-1RN exon 1ic (1868); IL-1RN exon 1ic (1887); Pic (1731). Genotyping of these markers could proceed as described in Clay et al., Hum. Genet. 97:723-26, 1996. PCRs could be performed using 5 μg genomic DNA in a final reaction volume of 250 μl containing 250 pmol forward and reverse primers and 1.5 mM MgCl2. The annealing temperature could be 57°C. Primers for exon 1ic PCR and sequencing could be:

5' TTACGCAGATAAGAACCAGTTTGG 3' (SEQ ID No. 21)

5' TTTCCTGGACGCTTGCTCACCAG 3' (SEQ ID No. 22)

The resulting product would be 426 bp, and the forward primer could be biotinylated to allow for ready sequencing.

Please replace the paragraph on page 38 and beginning with "TNF (-308)" with the following substitute paragraph:

TNF (-308): Cycling: [50° C, 2 min] x 1; [95° C, 10 min] x 1; [95° C, 15 sec, 58° C, 1 min] x 40; [15° C, hold]

Probe 1 5' - A (- TET) CCCCGTCCCCATGCCC (- TAMRA) -3' (SEQ ID No. 23)

Probe 2 5' - A (- FAM) ACCCCGTCCTCATGCCCC (- TAMRA) -3' (SEQ ID No. 24)

Forward 5' - GGCCACTGACTGATTTGTGTG T -3' (SEQ ID No. 25)

Reverse 5' - CAAAAGAAATGGAGGCAATAGGTT -3' (SEQ ID No. 26)

Please replace the paragraph running from page 38 to 39 and beginning with "TNF (-308)" with the following substitute paragraph:

TNF (-238): This single base variation in the TNFA promoter was described by D'Alfonso et al. In 1993 (D'Alfonso, S. and Richiardi, P.M. (1994) Immunogenetics 39:150-154). One of the PCR primers has a base change to create an *AvaII* site when amplifying allele 1.

Primers:

5' -GAA.GCC.CCT.CCC.AGT.TCT.AGT.TC-3' (-425/-403) (SEQ ID No. 27)

5' -CAC.TCC.CCA.TCC.TCC.CTG.GTC-3' (-236/-217) (SEQ ID No. 28)

Please replace the paragraph on page 39 and beginning with "IL-13 (+2581) G/A (Exon 4)" with the following substitute paragraph:

IL-13 (+2581) G/A (Exon 4): Allele 1 is a G, while allele 2 is an A. The presence of the A in allele 2 creates a site for the enzyme Nhe I (GCTAGC). Thus these alleles may be distinguished by amplifying the surrounding DNA and digesting with NheI.

PCR conditions:

forward primer 5' CCA GAC ATG TGG TGG GAC AGG G 3' (1741) (SEQ ID No. 29)

reverse primer 5' CGA GGC CCC AGG ACC CCA GTG AGC TAG CAG 3' (1742). (SEQ ID No. 30)

AMENDMENTS TO THE SPECIFICATION SHOWN WITH BRACKETS AND UNDERLINES:

In the paragraph on page 34 beginning with "IL-1RN (+2018)":

IL-1RN (+2018). PCR primers are designed (mismatched to the genomic sequence) to engineer two enzyme cutting sites on the two alleles to allow for RFLP analysis. The gene accesion number is X64532. Oligonucleotide primers are:

5' CTATCTGAGGAACAACCAACTAGTAGC 3' (SEQ ID No. 1 [7])

5' TAGGACATTGCACCTAGGGTTTGT 3' (SEQ ID No. 2 [8])

Cycling is performed at [96°C, 1 min]; [94°C, 1 min; 57°C, 1 min; 70°C, 2 min;] x 35; [70°C, 5 min] x 1; 4°C. Each PCR reaction is divided in two 25 ul aliquots: to one is added 5 Units of Alu 1, to the other 5 Units of Msp 1, in addition to 3 ul of the specific 10X restriction buffer. Incubation is at 37°C overnight. Electrophoresis is by PAGE 9%.

In the paragraph running from page 34 to page 35 and beginning with "IL-1RN (VNTR)":

IL-1RN (VNTR). The IL1-RN (VNTR) marker may be genotyped in accordance with the following procedure. As indicated above, the two alleles of the IL1-RN (+2018) marker are >97% in linkage disequilibrium with the two most frequent alleles of IL-1RN (VNTR), which are allele 1 and allele 2. The gene accession number is X64532. The oligonucleotide primers used for PCR amplification are:

5' CTCAGCAACACTCCTAT 3' (SEQ ID No. 3 [5]) [5']

5' TCCTGGTCTGCAGGTAA 3' (SEQ ID No. 4 [6])

In the paragraph on page 35 and beginning with "IL-1A (-889)":

IL-1A (-889). The IL-1A (-889) marker may be genotyped in accordance with the following procedure. McDowell et al., Arthritis Rheum. 38:221-28, 1995. One of the PCR primers has a base change to create an Nco I site when amplifying allele 1 (C at -889) to allow for RFLP analysis. The gene accession number is X03833. The oligonucleotide primers used for PCR amplification are:

5' AAG CTT GTT CTA CCA CCT GAA CTA GGC 3' (SEQ ID No. 5)

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In the paragraph running from page 35 to page 36 and beginning with "IL-1A (+4845)":

IL-1A (+4845). The IL-1A (+4845) marker may be genotyped in accordance with the following procedure. The PCR primers create an Fnu 4H1 restriction site in allele 1 to allow for RFLP analysis. The gene accession number is X03833. The oligonucleotide primers used for PCR amplification are:

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In the paragraph on page 36 and beginning with "IL-1B (-511)":

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5' TAAAATCAAGTGTTGATGTAG 3' (SEQ ID No. 14)

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5' TTAGTATTGCTGGTAGTATTCATAT 3' (SEQ ID No. 16)

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IL-13 (+2581) G/A (Exon 4): Allele 1 is a G, while allele 2 is an A. The presence of the A in allele 2 creates a site for the enzyme Nhe I (GCTAGC). Thus these alleles may be distinguished by amplifying the surrounding DNA and digesting with NheI.

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reverse primer 5' CGA GGC CCC AGG ACC CCA GTG AGC TAG CAG 3' (1742). (SEQ ID No. 30)

Conclusion

If there are any fees, due in connection with the filing of this Preliminary Amendment, please charge the fees to our Deposit Account No. 06-1448. If a fee is required for an extension of time under 37 C.F.R. §1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,
FOLEY, HOAG, & ELIOT

November 21, 2001

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